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QUALITY ASSESSMENT OF SELECTED *WITHANIA SOMNIFERA*-CONTAINING HERBAL FORMULATIONS USING GAS CHROMATOGRAPHY

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Abstract

The renewed significant interest in medicinal plants which hold an extensive therapeutic history has resulted in the need for the assessment of their quality using modern and sophisticated methods of their processing and usage. Therefore, quality assurance and control is of high priority to warrant the efficacy and consistency of herbal products. One of the challenges experienced by herbal products arises due to a lack of complete evaluation and with the use of herbal remedies on the rise; this then raises a question of quality within the manufactured capsules and evidence of the clinical efficacy of the remedies (Soni *et al.,* 2010). In order to assess the differences in quality between the different manufacturers found on the South African market – nine samples of herbal capsules and tablets containing *Withania somnifera* as a single or polyherbal formulation from seven different companies were sourced off the shelves from pharmacies and health shops. The samples were chosen based on high-ranking sales in the market, high consumer demand and industry popularity. The mass of each of the samples from each group weighed and compared to the mass of each capsule as stipulated on the packaging. The colours were examined with the naked eye and recorded. The powders were dissolved in the reagent for at least 72 hours. An Agilent Technologies Cary 60 UV-Visible double beam spectrophotometer with matched quartz cells was used for absorbance measurements. A gas chromatograph which is hyphenated to a mass spectrometer was used for analysis of the *Withania somnifera* containing formulations. It may be

deduced from the factors evaluated (mass, colour, presence of active ingredient and consistency) that these product lines appear to provide products containing the claimed active. However, further research regarding the quality of these products is warranted.

Keywords: *Withania somnifera*, ashwagandha, gas chromatography, quality, South Africa.

Introduction

Since time immemorial people looked for drugs in nature in search for treatment for their diseases. The initial stages of medicinal plants' use were instinctive (Pan et al., 2014). In time, the reasons for the usage of specific medicinal plants for treating certain ailments were being discovered (Padalia, 2012). Herbal medicines are produced from substances of herbal/ plant origin which are usually taken from various commercial and geographical sources. Thus, due to the potential lack of standardized conditions in which the original plants were grown under, the resultant properties and composition of active ingredients of the plants may be variable. For this reason, applying good manufacturing practice in the manufacturing of herbal medicines becomes an important tool for quality assurance (WHO, 2007).

Herbal preparations are commonly comprised of variable and complex components of different species or one and the same species, the composition and number of chemical compounds vary significantly. Due to an increase in questions about the quality of herbal preparations, there is a rising concern about what herbal products are constituted of (Brinker, 2014). Active plant identification and standardization of biologically active compounds in a herb is an important requirement for quality control and dose determination for plant-based medicines (Dubey et al., 2014).

Analytical techniques assist as a specific tool in the research of herbal drugs, allowing manufacturers to meet quality standards and specifications to seek marketing approval from regulatory authorities for therapeutic safety, efficacy and shelf-life of herbal drugs. The main objective of standardization of medicinal plants, amongst other reasons is to ensure therapeutic efficacy. Hence, maintenance of quality of these plant products is a crucial factor (Viswaroopan and Shailaja, 2016).

For the analysis of herbal extracts or products, the selection of equipment to be used is dependent on the purpose of the analysis and the properties of compounds to be evaluated and analysed in the herbal extract. Chromatography tools are used for isolation purposes. Certain equipment is more suitable for qualitative rather than quantitative analysis. Gas Chromatography (GC) and Ultraviolet (UV) are widely applied for herbal studies; not only are they used for identification and quantification of compounds in herbal extracts, but they are also used for comparison of chromatogram fingerprints of herbal products (Liu, 2015). Since plant extracts generally occur in combination with various types of bioactive compounds or active ingredients with the different polarities, separation of these phytochemicals is still a challenge for the identification process and characterization of bioactive compounds. Separation techniques is used to identify bioactive compounds (Naneu, 2014). GC was used as it offers enhanced sample identification, increased sensitivity, a higher range of analyzable samples, and more rapid results, which enable a whole new range of applications for GC-MS in many areas. It can therefore be concluded that automated GC-MS systems provide rapid and reproducible results in many applications (Cheriyedath, 2016) Within the South African market, the number of products that comply with the intended levels of quality is unclear.

Material and Methods

Research samples

From these products, nine samples of herbal capsules and tablets containing *Withania somnifera* as a single or polyherbal formulation were sourced off the shelves from pharmacies and health shops - from seven different companies. From the nine samples, six are locally produced products and the other three internationally produced products. Of the nine products, five are single formulations and the remaining four are polyherbal formulations; three samples of each are sourced from three different batches where available and some samples from either one or two batches. The samples were chosen based on high-ranking sales in the market, high consumer demand, industry popularity and they are easily accessible to the public. The nine samples were labeled according to the groups they fall under. The coding system was done according to groups of three even though some suppliers only had two samples. All the samples were emptied into identical glass vials and randomized when labeled to blind the researcher. One gram of each

powder was weighed and further emptied into separate vials. The laboratory assistant labeled the samples in a precise manner so that they could be correctly and easily tracked. The reagents used were chloroform, hexane and methanol, and were obtained Sigma Aldrich.

Sample Masses

One gram of each sample was weighed into a sample vial. Capsules were opened and emptied. Tablets were crushed to a fine powder. The colour of each batch and consistency of each tablet or capsule mass was observed. The mass of each of the samples from each group weighed and compared to the mass of each capsule as stipulated on the packaging.

Visual Analysis

One gram of each sample was weighed and emptied into medium or large vials. The colours were examined with the naked eye and recorded. The vials were then filled with 30 ml of methanol, hexane and chloroform and labeled. Regardless of the solvent used, the colours of the solution do not change.

Ultraviolet

One gram of the sample was dissolved in 15 ml methanol and shaken well. Then 35 ml of methanol was added to adjust the volume up to 50 ml. The powders were dissolved in the reagent for at least 72 hours. An Agilent Technologies Cary 60 UV-Visible double beam spectrophotometer with matched quartz cells was used for absorbance measurements. The software for the UV-Vis for solution was scanned in the range of 0 to 800 nm for maximum absorbance with cells of 1 cm length against the same solvents (methanol, hexane and chloroform) used as a blank.

Test Preparation: The dissolved 50 ml preparation was used for testing. From that, 1 ml of solution was withdrawn and put in a cuvette, the volume was adjusted with diluent of up to 10 ml. The cuvette was cleaned on the transparent sides with paper to ensure there are no fingerprints or residue on the cuvette and held on the opaque sides. The cuvette was placed in the spectrometer and the lid was closed. The UV-Vis recorded the graph and the graphs were plotted, wavelength versus absorbance for maximum peaks for all three solvents.

Gas Chromatography

For each A-1 vial, one gram of the powder was further weighed into three different vials for extraction in methanol, hexane and chloroform and labeled accordingly, i.e. A-1 (MOH), A-1 (Hexane) and A-1 (Chloroform). The powders were dissolved in the reagent for at least 72 hours. 1.5 microlitre's was filtered and stored in a vial for GC preparation. With the use of a rotavapor, the solvent extract was concentrated under reduced pressure at 40 degrees Celsius, suspended in water and sequentially separated with n-hexane, chloroform and methanol as per standard procedure.

A gas chromatograph which is hyphenated to a mass spectrometer and prepared with an auto-injector and auto-sampler was used for analysis of the *Withania somnifera* containing formulations. For analysis with gas chromatography, it was required that the sample was volatile. In order to ensure that the compounds of *Withania somnifera* are volatile, derivatisation of the formulation was essential before the formulations were analysed using GC-MS. Helium was used as the carrier gas. A capillary column was used for all chromatographic separations with these specifications: length, 30m; thickness, 0.250 micro meters, ID; 0.35mm and to be treated with polyethylene glycol. Other conditions required by GC-MS are ion source temperature of 250 degrees Celsius, interface temperature of 240 degrees Celsius, pressure of 100 kPa and the solvent cut time of 1.6 minutes. The GC column was injected with one microlitre of the sample. Operation of the injector was by means of a split mode with split ratio of 1:50 with a specific injection temperature. The column temperature program was directly proportional to the time period in minutes. The entire process of extraction took approximately 45 minutes (Uddin *et al*., 2013).

Results and Discussion

Sample Masses

Even with warranted attempts of accuracy, differences in the dosage amounts and errors may arise. Variations between samples even from the same bulk can occur. Variations in content of some active ingredients in herbal formulations may occur and these fluctuations in the amount of active ingredients may impact the proportions of ingredients present in the finished product (Kaul *et al*, 2016). According to the Medicines Control Council (MCC), this variability should not exceed 10% (MCC, 2016). According to the European pharmacopoeia the permitted variability at any given

time point should not exceed ± 10% of the labelled content of herbal substance or herbal preparation, unless a wider range is supported by a bioequivalence study (European pharmacopeia, 2011). Not all samples complied with the mass stipulations as noted in the table below. These variations in tablets may have arised due to factors such as the flowing properties of the powder, the pressure used in compression, the type of machines used in tableting as well as the speed of the tableting machine. The variations in weight of capsules may be explained by a possible defect of the capsule filling machine or a misalignment of the upper and lower capsule segments (Dshravani, 2013).

Table 2.1: Masses (Average) of each capsule on packaging in comparison to actual mass found.

Group Number	**Mass stated on packaging**	**Actual mass of each formulation**	**Mass specifications met? Yes/No**
1	400 mg	467 mg	Yes
2	969 mg	510 mg	No
3	250 mg	394 mg	Yes
4	850 mg	445 mg	No
5	500 mg	396 mg	Yes
6	*	403 mg	*
7	500 mg	602 mg	Yes
8	985 mg	495 mg	No
9	350 mg	286 mg	Yes

*****No mass was stated on the packaging of the product to determine the amounts of each herb in the formulation.

Visual Analysis

The visual assessment included the comparison of colour between two powders within the same batch and the comparison of colour of the powders between different batches of the same supplier (Efferth and Greten, 2012). The standard colour of *Withania somnifera* is a light shade colour, ranging from cream white to a yellowish brown colour (Agrawal *et al*., 2014). All groups appeared to have no colour variations when

comparing samples of the same supplier except for the two samples of group 5 from the same supplier, as noted below. Variations in colour may occur due to many reasons apart from the quality of the plant. Generally, the variations may be due to the age of the plant, time of harvest and the presence of active compounds within the plant as well as the agricultural and collection practices of the plants including the harvesting, transport and storage (Allam and Kumar, 2011).

Table 2.2: Colours recorded of each sample powder.

Group Number	Visual appearance of powder
1	Golden brown
2	Charcoal
3	Brown
4	Golden yellow
5	Deep Brown* and Cream white*
6	Brown
7	Cream white
8	Deep brown
9	Brown

*An exponential difference was detected in colour in both products.

(a) Sample D-3 with a deep brown colour **(b)** Sample E-1 with a cream white colour

Figure 2.1: Colour of powders from samples in Group 5.

Ultraviolet – vis (UV-vis)

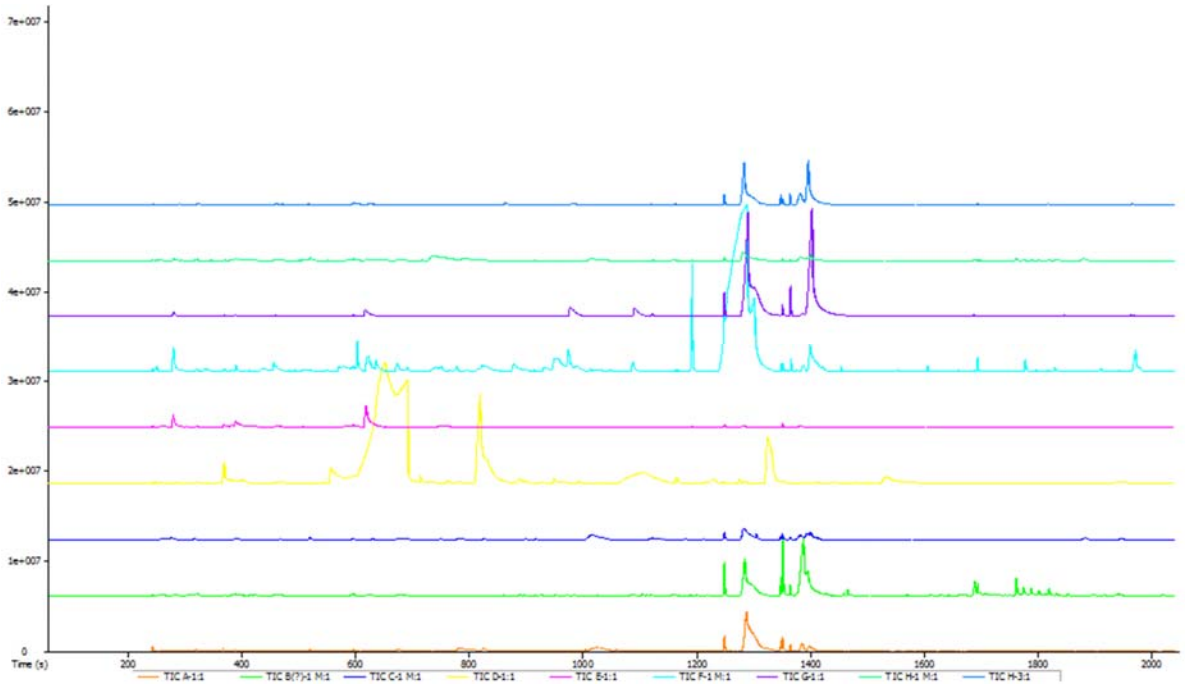
All groups were observed under all three solvents (hexane, chloroform and methanol) with the solvents used as a blank. The absorption profiles of all samples appeared to be most intense with maximum

absorption in the region between 650 nm and 750 nm. Specific absorbance of active ingredient, withanolide A was found roughly between the region of 200 nm and 250 nm for some samples and other samples did not exhibit any peaks depending on the solvents used for extraction.

Gas Chromatography (GC)

Only methanol profiles were used to compare the differences of the batches within the same supplier; this is due to the high polarity of methanol. The comparison of the different batches was based on the fact that the same amount of sample powder was weighed, the same amount of solvent was used as a diluent and the same processes of extraction were used to detect the presence of active ingredients. Withanolide as the active ingredient was found to be present if the peak was detected between the ranges of 1716.5 – 2000. As noted in some graphs, regardless how minute the peak is, this still shows the presence of the active ingredient. Ultimately, the presence of the active ingredient within each batch of all groups was validated using UV.

Figure 2.2: GC-MS Spectra Summation of all Suppliers (GC-MS Spectra of groups 1-9).



Conclusion

While considering the purpose of this study as well as the limited quantity of formulations used in the study, it can be deduced that the quality parameters investigated for both the international and locally produced formulations are acceptable. Due to these limitations, no conclusion can be reached as to whether the formulations complied to the standards set out by Good Manufacturing Practice (GMP) even though consistency was achieved from batch to batch. Therefore, further detailed evaluations of the precise quantification of the active ingredient, presence of any potential contaminant, as well as the comparisons between polyherbal and singular formulations is warranted.

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References

Agrawal, R., Upadhyay, A., Nayak, P. (2014). Influence of Drying on the Quality of Ashwagandha (Withania Somnifera). *Journal of Food and Pharmaceutical Sciences,* 2(1), pp. 63-67.

Allam, K., Kumar, G. (2011). Colorants - The Cosmetics for the Pharmaceutical Dosage Form. *International Journal of Pharmacy and Pharmaceutical Sciences,* 3(3), pp. 13-21.

Brinker, F. (2014). *Complex Herbs- Complete Medicines.* 1st ed. Oregon: Eclectic Medical Publications., pp. 22-25.

Cheriyedath, S. (2016). *Gas Chromatography- Mass Spectrometry (GC-MS) Applications.* [Online]
Available at: http://www.news-medical.net/life-sciences/Gas-Chromatography-Mass-Spectrometry-(GC-MS)-Applications.asp
[Accessed 12 March 2017].

Dshravani, (2013). *Quality Control of Capsules.* [Online]
Available at: http://www.pharmainfo.net/quality-control-capsules
[Accessed 27 November 2017].

Dubey, N., Kumar, R. and Tripathi, P. (2014). Global Promotion of Herbal Medicine: India's Opportunity. *Current Science,* 86(1), pp. 37-49.

Efferth, T. and Greten, H. (2012). Quality Control for Medicinal Plants. *Medicinal and Aromatic Plants,* 1(7), pp. 1-3.

European Pharmacopoeia. (2011). Guideline on Specification: Test Procedures and Acceptance Criteria for Herbal Substances, Herbal Preparations and Herbal Medicinal Products/ Traditional Medicinal Products. *Science Medicines Health.*, pp. 17-25.

Kaul, S., Ishida, Y., Tamura, K., Wada, T., Iitsuka, T., Garg, S. (2016). Novel Methods to Generate Active Ingredients-Enriched Ashwagandha Leaves and Extracts. *PLoS ONE,* 11(12), pp. 1-15.

Liu, W. (2015). *Traditional Herbal Medicine Research Methods.* 1st ed. Singapore: John Wiley and Sons, Inc., pp. 89.

Medicines Control Council (MCC). (2016). *Complementary Medicines - Health Supplements Safety and Efficacy.* 2nd ed. Pretoria: Department of Health., pp 21.

Naneu, M. (2014). *Proper Research of Traditional Medicinal Plants and Their Uses in the Masaai Community of Kenya.* 1st ed. Nairobi: Department of Biochemistry., pp.30.

Padalia, R. (2012). *Medicinal and Aromatic Plants: Chemical Goldmines.* 1st ed. New York: Medicinal Aromatic Plants., pp.43.

Pan, S., Litscher, G., Gao, S., Zhou, S., Yu, Z., Chen, H., Zhang, S., Tang, M., Sun, N., Ko, K. (2014). Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources. *Evidence-Based Complementary and Alternative Medicine,* 1(1), pp. 1-20.

Soni, H., Ribadiya, N., Bhatt, S., Sheth, R. (2010). Evaluation of Herbal Formulation (Capsule) containing Ashwagandha as a Single Herb with their Nutritional Value Determination. *International Journal of Applied Biology and Pharmaceutical Technology,* 1(3), pp. 960-967.

Uddin, G., Rauf, A., Gul, S., Saleem, M. (2013). Proximate Chemical Composition and Biological Profile of Fatty Acids of Withania Somnifera L. Dunal. *Journal of Medicinal Plants Research,* 7(27), pp. 2034-2039.

Viswaroopan, D., Arun Raj, G., Shailaja, U. (2016). Standardization of Ashwagandha Ghrita: A Herbal Ghee Based Ayurvedic Medicinal Preparation. *International Journal of Pharmaceutical Sciences and Research,* 7(2), pp. 819-823.

WHO, (2007). *WHO Guidelines on Good Manufacturing Practices (GMP) for Medicinal Plants.* 1st ed. France: WHO Press., pp.15-19.